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Pflugmacher, Stephan

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Translocation of the cyanobacterial toxin microcystin-LR into guttation drops of *Triticum aestivum* and remaining toxicity

Stephan Pflugmacher[1,2,3,*], Amalia Sulk[1,2], Sanghun Kim[4], Maranda Esterhuizen-Londt[1,2,3]

[1)] University of Helsinki, Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, Aquatic Ecotoxicology in an Urban Environment, Niemenkatu 73, 15140 Lahti, Finland

[2)] Joint Laboratory of Applied Ecotoxicology, Environmental Safety Group, Korea Institute of Science and Technology Europe (KIST Europe) Forschungsgesellschaft mbH, Universität des Saarlandes Campus E7 1, Saarbrücken, 66123, Germany

[3)] Helsinki Institute of Sustainability (HELSUS), Fabianinkatu 33, 00014 Helsinki

[4)] Kyung-sung University, Department of Pharmaceutical Science and Technology, 191, Jeongjail-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13557 Republic of Korea

*Corresponding author: Stephan Pflugmacher, University of Helsinki, Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, Aquatic Ecotoxicology in an Urban Environment, Niemenkatu 73, 15140 Lahti, Finland, Tel: +35850 316 7329, Email: stephan.pflugmacher@helsinki.fi

Highlights

- Microcystin-LR was taken up *via* the roots of *Triticum aestivum.*
- MC-LR could be detected in the roots, stems, and leaves.
- MC-LR occurred in xylem sap, and guttation drops at the same concentration.
- MC-LR was detected in and thus is transported *via* the xylem.
- MC-LR at the concentration occurring in the guttation drops is not toxic to daphids.

Capsule

Microcystin was taken up by Triticum aestivum (wheat) after exposure via irrigation, transported through the roots to the stems and leaves via the xylem, and could be detected in the guttation drops.

Abstract

Uptake of the commonly occurring cyanobacterial toxin microcystin-LR (MC-LR) into crop plants *via* spray irrigation has been demonstrated. As other hazardous compounds such as pesticides were shown to be transported within plants, it was essential to understand the transport and fate of MC-LR in plants and the risks posed to grazers and other consumers. Of specific interest was to investigate if MC-LR could be detected in guttation drops and the toxicity thereof. *Triticum aestivum* (wheat) seedlings were exposed to 100 µg L^{-1} MC-LR in two separate experiments during which guttation drops were collected at various time points. The plants of one experiment were sectioned to investigate MC-LR distribution to the various plant appendages *via* liquid chromatography-tandem mass spectrometry analysis. After exposure, MC-LR could be detected in the roots, stems, leaves, and the guttation drops. However, the guttation drops were not toxic to *Daphnia*. As the environmentally relevant toxin concentration used was not sufficient to promote mortality in *Daphnia*, the physiological effect in insects, which rely on guttation drops as a water source, remains unknown. Combined with other contaminants that insects may be exposed to, the additional MC-LR exposure could contribute to the overall toxicity through the "tears of death".

Keywords: Cyanobacterial toxins, guttation, microcystin-LR, translocation, *Triticum aestivum* (wheat)

1. Introduction

Plants are the initiation point for most food webs, thus forming a central part of our global ecosystem. They are a vital resource of food for humans and animals, the latter in turn again serving as human food resources. For many years our ecosystems have had to face a constant input of „xenobiotics“ (gr. xen(o) – strange; biosis – way of life), which are human-made compounds produced for a variety of purposes. Many of them are released deliberately, such as various pesticides in agricultural systems. The uptake of these organic pollutants into plants is usually a function of several chemical and physical properties they exhibit such as hydrophilicity, water solubility, and vapour pressure. The absorption depends on the respective pollutants and the environmental conditions such as temperature, UV-light, soil properties, plant species as well as plant health.

One of the critical factors for many physiological processes in plants is water. In plants, water is a solvent and a transport medium (for example for nutrients and photosynthetic products), is involved in numerous biochemical reactions and is responsible for turgor pressure and stomata opening. Therefore, after being taken up by millions of root hairs, which are thin-walled, slender extensions of the root epidermal cells, water moves freely from soil into the capillary spaces between the cortex in the roots. The water is then transported by a water gradient, with osmosis as the driving force. Once water is absorbed, there are two ways to move deeper into the plant, i.e. the apoplast pathway and symplast pathway (Hopkins, 1999). The apoplast pathway leads through the cell walls, which are freely permeable to small molecules. Through this pathway, the plant has no control over the movement of substances, as the cells are literally dead. However, coming to endodermis cells, the Casparian stripe effectively blocks this pathway, so all

77 substances including water have to enter the stele and thus xylem for transport through
78 the symplast.

79 Plants cannot discriminate between beneficial or harmful compounds, nor can they
80 distinguish between natural or xenobiotic substances. Unfortunately, most toxic
81 compounds behave the same way as nutrients if the physicochemical properties are
82 similar (Riederer, 1990). Transport within plants takes place in xylem and phloem. In
83 mature plants, the tracheids in the xylem fibrous cells are dead, whereas in phloem sieve
84 elements that have fully functional membranes are filled with living protoplasm.
85 Movement in phloem is achieved by active processes, which generally require ATP
86 (Trapp, 1995). Therefore, the transport of hydrophobic organic compounds is limited in
87 the phloem.

88 Plants do have possibilities to excrete solutes. One of these possibilities is called
89 guttation (lat. gutta = drop), which is the formation of drops exudated *via* xylem sap
90 through hydathodes, a process linked to root pressure (Slayter, 1967) and is in some
91 ways also derived from the transpiration stream (Fig. 1). As Klepper and Kaufmann
92 (1966) discovered, guttation fluid and xylem sap have different chemical compositions,
93 i.e. guttation fluid contains different kind of amino acids, sugars, and inorganic salts
94 (Goatley and Lewis, 1966; Sheldrake and Northcote, 1968) and even some active
95 enzymes can be detected in this fluid (Tab. 1) (Biles and Abeles, 1991; Kerstetter et al.,
96 1998; Komarnytsky et al., 2000). In other words, the generation of the guttation fluid
97 appears to be quite a selective process (Coupland and Caseley, 1979). In the past years,
98 more and more reports have shown the presence of xenobiotics (Tab. 1), such as
99 glyphosate, imidacloprid, or clothianidin in the guttation fluid (Girolami et al., 2009;
100 Tapparo et al., 2011). The last two mentioned substances, belonging to the group of

neonicotinoids, are hazardous for insects such as the honeybee (Ratnieks and Carrek, 2010).

Figure 1 here.

Cyanobacterial blooms in freshwater bodies are a global environmental problem due to promoted eutrophication and climate change (Scholz et al., 2017). As a result, the occurrence of toxic cyanobacterial blooms as well as the amount of cyanobacterial toxins, such as in the case of the hepatotoxic microcystins (Omidi et al., 2018), have become considerably increased. These toxins may have interactions with central components of aquatic ecosystems, i.e. aquatic plants causing adverse effects and disrupting the ecosystem (Pflugmacher, 2004). As some of the bodies of water are used for spray irrigation, cyanotoxins can be transferred into the food chain. The uptake of cyanobacterial toxins has been documented in terrestrial plants (Pflugmacher et al., 2006; Pflugmacher et al., 2007a and b; Contardo-Jara et al., 2018); however, its excretion during plant guttation has not been investigated. Thus, it was necessary to investigate the fate and transport of a commonly occurring cyanobacterial toxin in a terrestrial plant. As microcystin-LR (MC-LR) is so commonly detected and well studied (Omidi et al., 2018), it was chosen for this investigation. The present study, therefore, aimed to investigate the plant internal transport of the cyanobacterial toxin MC-LR after uptake *via* the root system. Furthermore, the possibility of MC-LR transfer into guttation drops was investigated. Guttation drops are an important water source for many insects, especially bees, and could negatively affect biodiversity indirectly. Therefore, the potential toxicity of the guttation drops was tested using the immobilisation test of the crustacea *Daphnia magna* as a sentinel for hazards stemming from guttation drops.

2. Materials and methods

2.1.Chemicals and reagents

All chemicals were analytical grade and purchased from Sigma Aldrich unless stated otherwise. MC-LR (HPLC-grade; purity ≥ 95%) isolated from *Microcystis aeruginosa* was obtained from Enzo Life Sciences (Germany). The toxin was suspended and diluted with HPLC-grade methanol (VWR International GmbH, France) to an MC-LR stock solution of 100 µg L^{-1} before storage at -20°C.

2.2.Wheat plant cultivation

Wheat (*Triticum aestivum* L. Variant: Taifun M) seeds from a local supplier (Ecocontrol, Osterrode, Germany) were left to imbibe in standard medium (900 mg L^{-1} KNO_3, 900 mg L^{-1} $Ca(NO_3)_2$, 360 mg L^{-1} $MgSO_4$, 200 mg L^{-1} KH_2PO_4, 40 mg L^{-1} Fe-EDTA and micronutrients: 1 mg L^{-1} $MnSO_4$, 0.2 mg L^{-1} $CuSO_4$, 0.2 mg L^{-1} $ZnSO_4$, 1.8 mg L^{-1} H_3BO_3, 3.4 mg L^{-1} $(NH_4)_6Mo_7O_{24}$, and 9 mg L^{-1} $CoCl_2$) (Walters and Kingham, 1990) for 1 h. Afterwards, the seeds were disinfected using first 0.1% Tween 80 solution (w/w), 0.5% sodium hypochlorite (w/w), and finally 0.75% hydrogen peroxide solution (w/w) and washed between these steps with distilled water. For germination, the seeds were transferred onto filter paper (Whatman No. 1), wetted with the standard medium in Petri dishes, and incubated at room temperature (21°C) in the dark for 24 h.

Seeds, from which the primary root protruded after 48 h, were transferred in single to reagent tubes filled two thirds with washed, sterilised (20 min, 121°C) sand in semi-hydroponic culture using the standard medium. Seeds were placed 0.5 – 1.0 cm deep into the sand for further growth and incubated at 25°C with a day/night cycle of 14 h:10

146 h (100 µE m^{-2} s^{-1}) in a commercially available greenhouse (4.6 m^2 size, Bauhaus, Berlin,
147 Germany) made of polycarbonate plates and aluminium frame.

148 *2.3.Collection of guttation liquid*

149 To initiate guttation of the seedlings, the reagent tubes were placed in trays with water to
150 maintain a high humidity of 95%. When the seedling shoots were up to 3 cm tall after 6
151 d, guttation liquid was collected twice daily for the following 3 d, drop by drop from the
152 hydathodes around of the primary leaf using a glass Pasteur pipette before the exposures
153 commenced. Droplets had an average size 1.35 ± 0.14 mm (*n* = 800) and the average
154 volume per wheat hydathode per night was 0.8 ± 0.3 x 10^{-7} L. After sampling the
155 guttation drops collected were immediately frozen in liquid nitrogen and stored at -
156 80°C. Guttation liquid exhibit a pH of 5.1 ± 0.1.

157 *2.4.Exposure scenario*

158 2.4.1. Transfer of toxin into plant sections

159 After 6 d of growth, a total of 1000 seedlings, separated in five independent batches
160 with 200 seedlings each, were obtained. These batches were again separated into five
161 independent batches with 25 seedlings each. Each seedling was irrigated daily for 7 d
162 with water (1000 µL per seedling) containing 100 µg L^{-1} MC-LR (0.7 µg MC-LR per
163 seedling). As an independent control, 100 seedlings were irrigated with water without
164 toxin. The seedlings were collected after 0 h, 2 h, 4 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144
165 h, and 168 h. The seedlings were washed with methanol and water in three successions
166 to remove any possible surface bound MC-LR on the roots and then separated into root,
167 stem, and leaf sections. Guttation drops were sampled twice a day using glass Pasteur
168 pipettes for 7 d. Guttation drop samples were combined from each batch from exposure

as well as the controls separately for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (described in section 2.5).

2.4.2. Toxin content in guttation drops and xylem sap

After 6 d of growth, a total of 500 seedlings (divided into 5 batches) were each exposed to 1000 µL of water containing 100 µg L^{-1} MC-LR (0.1 µg) for 96 h and 168 h respectively before sampling. As a control, 100 seedlings in total were irrigated with water without toxin. For xylem sap sampling, the seedlings were cut 1.0 cm above the sand surface using a sharp scalpel to collect xylem sap coming from the root system directly. Xylem sap was collected using a glass Pasteur pipette and combined from all seedlings of one batch in exposure as well as control samples, separately.

2.4.3. Toxicity of guttation drops

In order to investigate the potential toxicity of the guttation drops, the *Daphnia* sp. acute immobilisation test according to ISO 6341:2012 (2012) was performed. *Daphnia magna* Strauss from an established laboratory culture was used. To set up the test, young *D. magna* aged less than 24 h were exposed to the guttation drops in various concentrations for a period of 48 h. The immobilisation was recorded after 48 h and compared with control values. The dilutions were prepared in standard water (294 mg·L^{-1} $CaCl_2 \cdot H_2O$, 123 mg·L^{-1} $MgSO_4 \cdot 7H_2O$, 63.0 mg·L^{-1} $NaHCO_3$, and 5.50 mg·L^{-1} KCl) at a pH of 7.0. For each concentration tested, in total 20 animals were divided into four groups, i.e. five animals were used per treatment replicate, the control and positive control. The exposure temperature was 19 ± 1°C and a 16 h:8 h light/dark cycle was applied. Test vessels were not aerated during the test, nor was any food supplied to the *D. magna* during the test.

192 *2.5.Microcystin-LR analysis*

193 To reach detectable MC-LR concentrations for toxin analysis *via* LC-MS/MS, a pre-
194 concentration of MC-LR *via* solid-phase-extraction (SPE) was done using reversed-
195 phase cartridges (Sep-Pak® tC18, 400 mg sorbent, Waters, Ireland). The sample was
196 passed through the SPE tube for toxin enrichment followed by eluting with 5 mL of
197 99% methanol (Carl Roth, Germany). Subsequently, all methanol was removed in a
198 vacuum centrifuge (Concentrator plus/Vacufuge® plus, Eppendorf AG, Germany) at a
199 temperature of 30°C and finally resuspended in 500 µL HPLC-grade methanol
200 (Spengler et al., 2015).

201 MC-LR quantification was performed by LC-MS/MS (Alliance 2695 UHPLC combined
202 with a Micromass Quattro microTM, Waters) using the reverse phase column Kinetex™
203 C18 (2.1 mm × 50 mm, 2.6 µm pore size, Phenomenex, USA). The column oven
204 temperature was set at 40°C with an injection volume of 20 µL. The mobile phase
205 consisted of solution A (Milli-Q water containing 0.1% trifluoroacetic acid (TFA) and
206 5% acetonitrile (ACN)) and solution B (ACN containing 0.1% TFA) at a flow rate of
207 0.2 mL min^{-1}. A gradient was generated between both solutions. The gradient conditions
208 (solution A: solution B) were 65:35 at 3 min, 35:65 from 3.75 to 7 min and 0:100 from
209 7.8 to 12 min. An elution peak for MC-LR was observed at 7.44 min. Mass spectral data
210 analyses were performed using electrospray ionisation (ESI) in a positive ion mode with
211 a collision energy of 65 V. Desolvation gas N_2 was set as trigger gas and argon as the
212 collision gas. For the subsequent MS/MS detection, the MRM mode was used with a
213 mass transfer of 995.5 (Q1) and 107.3, 135.1, 213.2 and 357.2 (Q3) for MC-LR.
214 Calibration was linear (r^2 = 0.999) between 5 and 500 µg L^{-1}. The LOD was 1 µg $L^{-1,}$
215 and LOQ was 2 µg L^{-1} (Contardo-Jara et al., 2015).

216

2.6.Statistical Tests

SPSS was used to perform a descriptive analysis based on the mean of toxin content (Arbuckle, 2010). Results are expressed as mean ± standard deviation (SD). Data were submitted to one-way analysis of variance (ANOVA). When the overall F statistic was significant, pair-wise comparisons were performed by Tukey–Kramer test (Sokal and Rohlf, 1997). An alpha value of 0.05 level was set for significance.

3. Results and Discussion

Exposure of agricultural plants *via* spray irrigation to water containing cyanobacterial toxins have severe effects on plant physiology and therefore also have economic consequences (Codd et al., 1999; McElhiney et al., 2001). Accumulation of cyanotoxins in plants *via* spray irrigation as the source has been reported previously (Pflugmacher et al., 2007a and b; Peuthert et al., 2007; Lahrouni et al., 2015; Contardo-Jara et al., 2018). However, the fate of these toxins within the plant remains poorly understood. As seen for *Phragmites australis*, the MC-LR is taken up by plant root systems and internally transported into stem and leaves (Pflugmacher et al., 2001). Based on this result, long-distance transport of the polar molecule MC-LR *via* the xylem becomes a plausible scenario and was thus investigated. For the pesticide imidacloprid, an acropetal xylem transport was shown in *Citrus sinensis*, after application of the pesticide to the tree bark (Mendel et al., 2000). According to Rudolph-Böhmer et al. (1994), MC-LR contains two ionizable carboxyl groups and one ionizable amino group outside the cyclic structure formed by peptide bonds. The pKa values of those groups ranged between 2.09 and 12.48 (Rudolph-Böhner et al., 1994). The pKa is one determinant for the ability to

240 penetrate biomembranes and responsibility for root uptake and translocation into the
241 xylem of a molecule (Sur and Stork, 2003).

242 *3.1.Transfer of toxin into plant sections*

243 In the present study, exposure of the seedlings to MC-LR led to an uptake of the toxin
244 into the plant (Fig. 2). As the only parts exposed to MC-LR, uptake occurred *via* the root
245 pathway. Uptake was time-dependent, starting with the detection of 1.4 ± 0.3 µg g^{-1} MC-
246 LR in the roots as soon as 2 h after exposure commenced, increasing exponentially (r^2 =
247 0.976) to a maximum concentration of 69.6 ± 7.4 µg g^{-1} after 168 h (Fig. 2A). As MC-
248 LR is a water-soluble polar compound, it can be assumed it entered the root dissolved in
249 water *via* the apoplastic pathway. However, it is likely that the hydrophobicity of the
250 Casparian stripe forced the dissolved MC-LR to transverse the plasma membrane to
251 enter the symplast where it likely entered, according to its chemical properties, the
252 xylem as it was detected in the stems (Fig 2B).

253 During the exposure period, the toxin moves through the stem (max. concentration after
254 168 h 40.9 ± 2.8 µg g^{-1}) (Fig. 2B) into the leaves of the seedlings (max. concentration of
255 toxin after 168 h 55.6 ± 6.6 µg g^{-1}) (Fig. 2C). Detecting MC-LR in the leaves of plants
256 of which only the roots were exposed to MC-LR, supports the hypothesis that MC-LR is
257 transported *via* the xylem. No toxin could be detected in the control plants that were
258 watered only with toxin-free water.

259 ***Figure 2 here.***

260 In a semi-field experiment using *T. aestivum* exposed to MC-LR and –RR as well as a
261 cell-free cyanobacterial crude extract individually for three months (0.5 µg L^{-1}), the
262 effects on root growth could be clearly shown (Pflugmacher et al., 2007b). However, in
263 the current study during which a substantially lower exposure concentration was used

(100 µg L^{-1}) for a shorter period (7 d), no adverse effects on root growth could be observed.

Figure 3 here.

In the guttation drops, MC-LR could already be detected after 48 h at a concentration of 4.6 ± 1.6 mg L^{-1} (Fig. 3). The toxin concentration in the guttation drops increased exponentially with time (r^2 = 0.979) to 60.0 ± 2.8 mg L^{-1} after 168 h of exposure. The detection of MC-LR in the guttation drops indicate an active transport of the toxin *via* the stems into the leaves and the guttation drops. When calculating the amount of MC-LR detected in the guttation liquid per seedling after 7 d, a total of 2.4 µg per seedling was reached. A similar calculation by Riebe (2009) showed that the amount of neonicotinoids in the guttation liquid per seedling was in the range of 0.5 - 1.3 mg. Those values refer to a whole growing season and not only to a 7 d growth experiment. Taking into account the effect of pooling all the guttation drops from the leaves of *T. aestivum* seedlings; this also may have led to a kind of dilution.

3.2.Toxin content in guttation drops and xylem sap

When comparing the toxin concentration of the xylem sap and guttation drop (Fig. 4), the transport is manifested. Guttation drops are exudates of the xylem sap. At night, stomata of plants are usually closed. Therefore no transpiration occurs. Root pressure builds up because water from soil moisture will force water to enter the roots. This root pressure forces water to exude through the hydathodes, forming guttation drops (Wilson, 1923). After 96 h of exposure, 19.3 ± 6.9 mg L^{-1} MC-LR could be measured in the xylem sap samples (Fig. 4). The toxin concentration in the guttation drops at the same time was 11.6 ± 2.2 mg L^{-1}, resembling 60 % of the xylem sap concentration. After 168 h of exposure, the MC-LR concentration in xylem sap reached 56.6 ± 9.1 mg L^{-1}, and

288 the concentration in the guttation drops reached 52.4 ± 7.2 mg L^{-1}. Typically, the
289 dissolved content of salts, sugars, amino acids, or even whole enzymes (catalase,
290 peroxidase) in the guttation drops (Wilson, 1923; Biles and Abeles, 1991) is very low
291 and usually below 1% (Pistorius et al., 2011). The amount of MC-LR detected in the
292 guttation drop after 168 h exposure was 0.024% of the total applied toxin amount of 100
293 µg L^{-1}.

294 ***Figure 4 here.***

295 Comparing the molecular sizes of compounds such as galactose (180.16 g mol^{-1}),
296 inositol (180.16 g mol^{-1}), asparagine (132.12 g mol^{-1}), glutamine (146.14 g mol^{-1}), and
297 methionine (149.21 g mol^{-1}) (Goatly and Lewis, 1966) or some pesticides such as
298 clothianidin (249.68 g mol^{-1}), diuron (233.10 g mol^{-1}) or chloramben (206.02 g mol^{-1})
299 (Stroller, 1970), previously detected in guttation drops, MC-LR (995.16 g mol^{-1}) is
300 significantly larger. However, as even larger biomolecules such as the enzymes catalase
301 (240,000 g mol^{-1}) or peroxidases (44,000 g mol^{-1}) are secreted into guttation drops, the
302 hepatotoxin is within the lower range. However, these enzymes are synthesised within
303 the plant cell, and MC-LR is taken up *via* the root from outside. This means that the
304 toxin has to overcome the Casparian strip barrier in order to enter the xylem sap. As
305 MC-LR could be detected in roots, stem, and leaf, the uptake mechanisms (transporters)
306 might be similar to amino acid and protein taken up into root cells (Lonhienne et al.,
307 2014). MC-LR consists of a ring of seven amino acids, so it can be speculated that
308 similar transporters are involved such as proton-amino acid symporters (Eriksson et al.,
309 1990). In addition, the uptake of MC-LR *via* pinocytic processes has been discussed as a
310 possible uptake route (Vesterkvist and Meriluoto, 2003).

311 According to Ortiz-Lopez et al. (2000), more than two dozen amino acid transporters in
312 plants are known, some of which might be active in bringing MC-LR into the root cells.

313 The concentration of toxin in the guttation drops was visually but not statistically lower
314 than that detected on xylem sap after both 96 and 168 h of exposure ($p > 0.05$);
315 nevertheless, the small difference indicated the removal of the toxin from the xylem sap
316 during the movement through the epithelium. This removal might be due to possible
317 biotransformation *via* conjugation by glutathione S-transferases as seen already in
318 different plants (Pflugmacher et al., 1998; 2001).

319 *3.3.Toxicity of guttation drops*

320 Detection of MC-LR in the guttation drops led to the question of whether this poses any
321 risk to insects using guttation drops as a water source. Guttation drops are for many
322 insects, including honeybees (Riebe, 2009), a potential source of water, even though this
323 source is available the whole day, but only in the morning and evenings, and even not
324 daily. Honeybees usually need water in the hive to regulate air humidity, to cool down
325 the temperature, and for the production of larval food (Johansson and Johansson, 1978);
326 however, there is no water storage in the hive. As the water collecting worker bees
327 might choose water sources near (around 50 m) the hive to avoid energy consuming
328 long-distance flights, an agricultural field nearby might present as the closest water
329 source (Kuehnholz and Seeley, 1997; Shawki et al., 2006).

330 To test the potential toxicity of guttation drops, the well-established ecotoxicological
331 test using *D. magna* immobilisation was performed. LC_{50} was calculated according to
332 Finney (1952) to be 1.48 ± 0.29 mg L^{-1} for the guttation drops. For MC-LR, the 48 h
333 LC_{50} reported in literature ranged from 1.78 to 6.86 mg L^{-1} (Sieroslawska, 2013; Piontek
334 and Czyzewska, 2017). Therefore, the toxicity of MC-LR secreted into the guttation
335 drops is still on the lower range of pure MC-LR tested. MC-LR was reported to be toxic
336 to terrestrial insects such as *Plutella xylostella* (diamond-backed moth), *Spodoptera*
337 *littoralis* (cotton leafworm), *Pieris brassicae* (cabbage white butterfly), and *Musca*

domestica (housefly). For *P. xylostella*, an LC_{50} 24 h of 1.02 µg toxin per cm^2 treated leaf surface was detected (Delaney and Wilkins, 1995). Other insects such as *Periplaneta americana* (American cockroach), *Tenebrio molitar* (Yellow mealworm), and *Gryllus bimaculatus bimaculatus* (common cricket) showed greater sensitivity towards MC-LR, which is toxic to them (Oberholster et al., 2009). However, further, more thorough toxicological testing of the guttation drops is necessary to evaluate any threats posed to insects.

4. Conclusion

After exposure, MC-LR could be detected in all three tested plant sections of *T. aestivum* seedlings and the guttation drops. As the environmentally relevant concentration of the toxin used in the present experiment was not sufficient to promote mortality in *D. magna*, it is still unknown how insects which rely on guttation drops as a source of water will react physiologically in nature. Combined with other contaminants that insects may be exposed to, this route of MC-LR exposure contributes to the overall toxicity through the guttation as the plant "tears of death".

Author contributions

SP, AS, SK and MEL designed the research; SP, AS performed the research; SP analysed the data, and SP and MEL wrote the paper. All authors read, gave comments, and approved the manuscript.

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Declaration of interest

The authors declare that there is no conflict of interest.

ORCID

Stephan Pflugmacher: https://orcid.org/0000-0003-1052-2905

Amalia Sulk: N/A

Sanghun Kim: https://orcid.org/0000-0002-2936-5084

Maranda Esterhuizen-Londt: https://orcid.org/0000-0002-2342-3941

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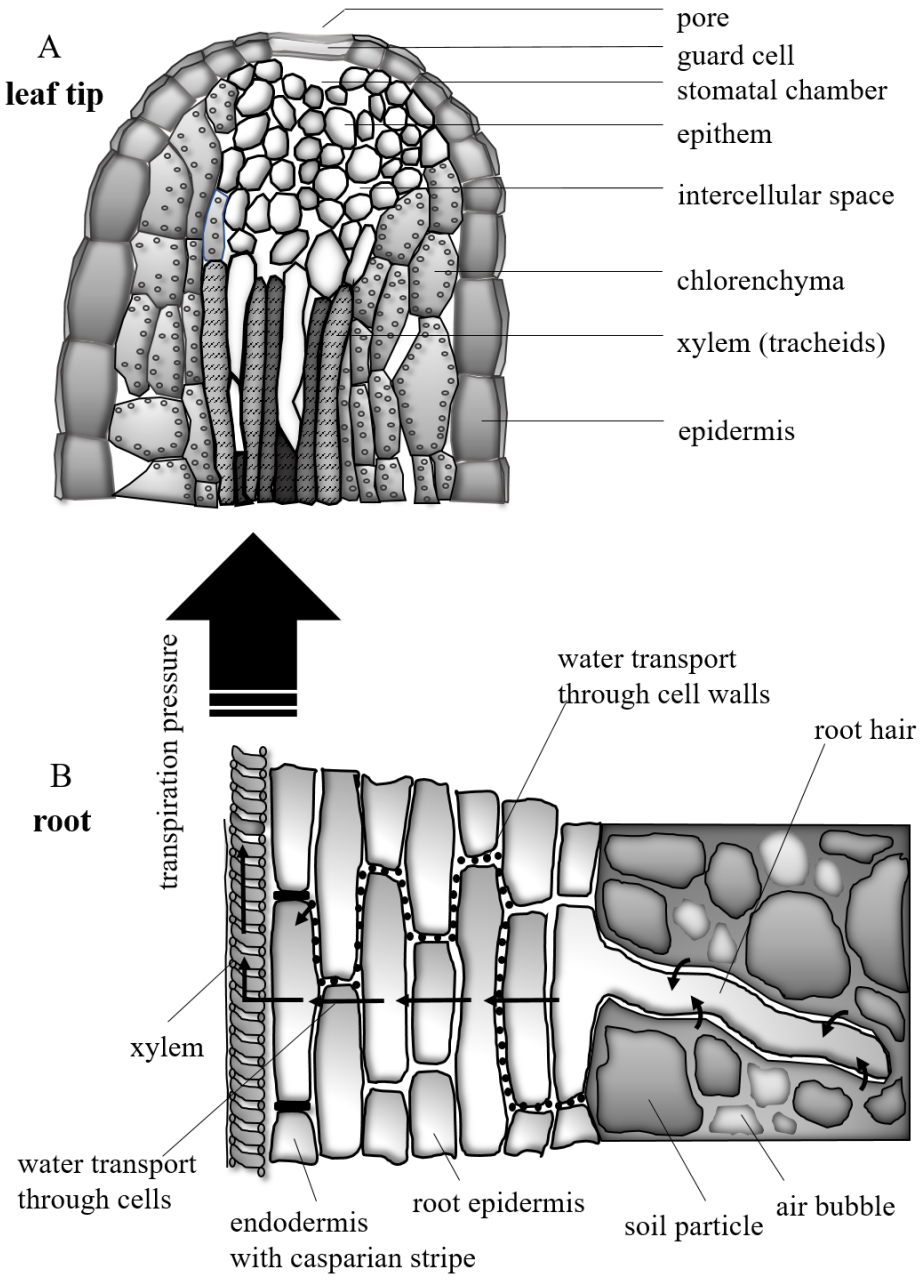
525 **Table 1:** Different compounds previously detected in guttation drops of different plants.
526 For pesticides, the concentrations were in the mg L^{-1} range for a more extended vegetation
527 period.

Compound/protein	Plant species	Concentration*	Reference
Clothianidin	*Zea mays*	8-102 mg L^{-1}	Frommberger et al., 2011 Tapparo et al., 2011 Girolami et al., 2009
Imidacloprid	*Zea mays*	346 mg L^{-1}	Tapparo et al., 2011 Girolami et al., 2009 Hoffmann and Castle, 2012 Larson et al., 2015
	Cucumis melo	48 mg L^{-1}	
	Agrostis stolonifera	37 mg L^{-1}	
Thiamethoxam	*Zea mays*	12-146 mg L^{-1}	Tapparo et al., 2011 Girolami et al., 2009
Glyphosate	*Agropyron repens*	NR	Coupland and Caseley (1979)
Catalase	*Zea mays*	NR	Wilson (1923) Biles and Abeles (1991) Komarnytsky et al. (2000)
	Avena sativa	NR	
Peroxidase	*Cynodon dactylon*	NR	Wilson (1923) Biles and Abeles (1991) Komarnytsky et al. (2000)
	Poa pratensis	NR	

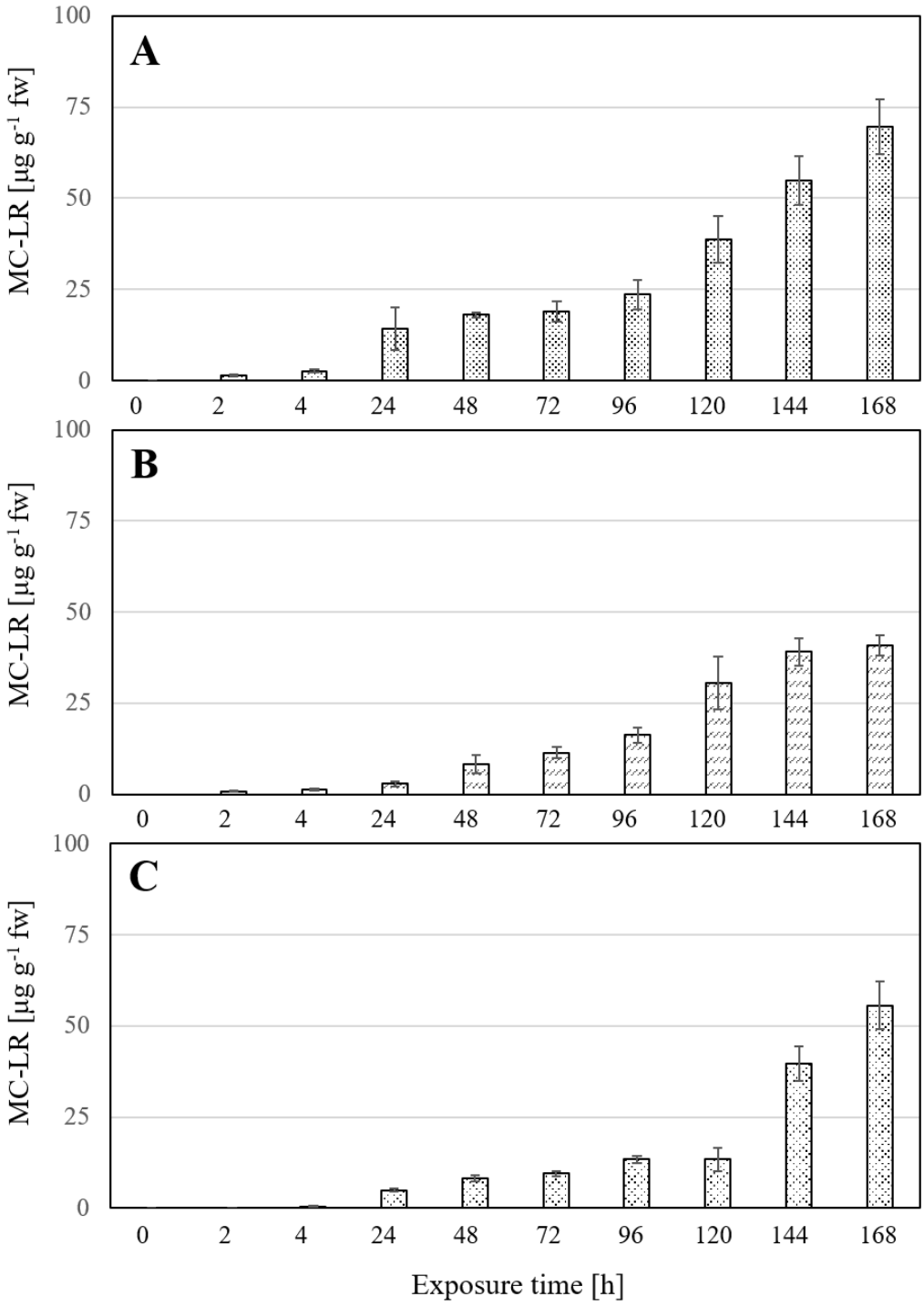
528 *NR: not reported

529 **Figure titles:**



530

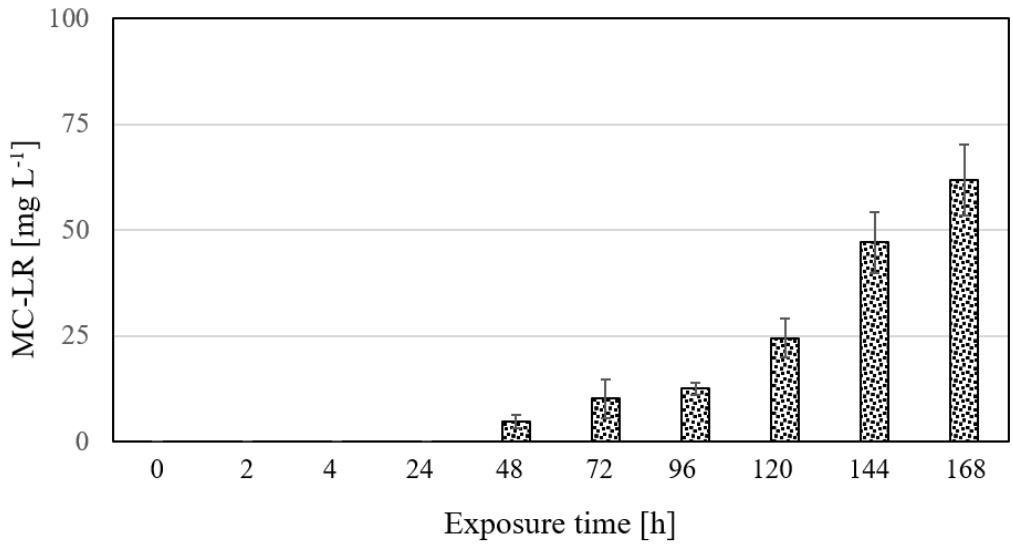
531 **Fig. 1:** Schematic drawing of the proposed uptake possibilities of water and dissolved
532 compounds including microcystin-LR *via* (B) root hair into the root system of plants
533 transporting water through cell walls and cells into the xylem. Arriving at the xylem, the
534 root pressure will facilitate the transport up into stem and leaf. At the (A) leaf tip, the
535 hydathode structure (redrawn from Stevens (1956)) will allow the water and solved
536 compounds to be excreted from the leaf tip as guttation drop.



537

538 **Fig. 2:** MC-LR concentration detected in (A) the root system (B) the stem, and (C) leaves
539 of the *T. aestivum* seedlings. Data represent the mean concentration of MC-LR in
540 microgram per gram of plant fresh weight ± the standard deviation (n = 200).

541

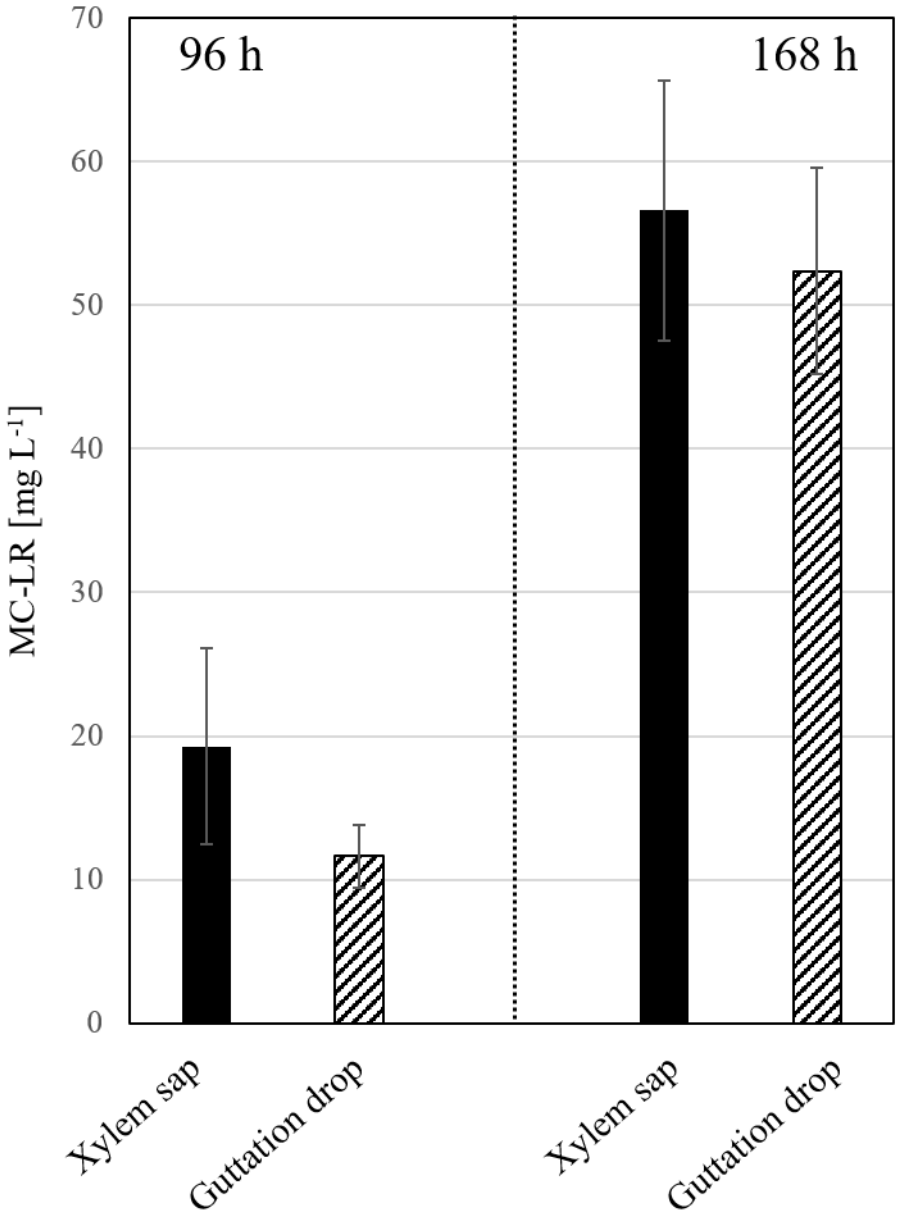


542

543 **Fig. 3:** MC-LR concentration in the guttation drops of *T. aestivum* after 48 h of exposure.

544 Data represent the mean concentration of MC-LR in milligram per litre guttation collected

545 ± the standard deviation (n = 200).



546

547 **Fig. 4:** Comparison of MC-LR concentration in xylem sap and guttation drops after 96 h
548 and 168 h of exposure. Data represent the mean concentration of MC-LR in milligram per
549 litre guttation collected ± standard deviation (*n* = 200).